PeptiGel® Protocol:

3D Cell Culture

Before you begin, please note:

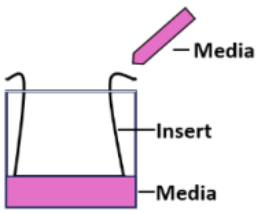
This protocol describes the use of PeptiGels® for 3-dimensional (3D) cell culture.

It's recommended to use a positive displacement pipette (such as the Gilson piston pipette) to allow easy pipetting as these are viscous hydrogels. Use of an air displacement pipette could lead to the introduction of bubbles to your PeptiGel®.

It's also recommended to use cell inserts (such as Greiner Bio-One Thincerts™ or equivalent) to increase gel stability and cell culture medium diffusion.

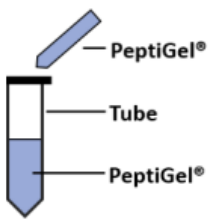
This protocol has been written for a total volume of 0.2 mL PeptiGel® with 24-well inserts as an example. Scale up or down according to culture requirements.

1. Pre-wet the inserts in cell culture medium/PBS for 1 hour to prevent bubbles getting trapped into the membrane pores.



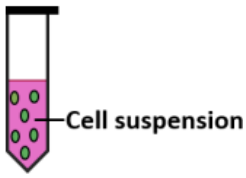
2. Remove PeptiGel® from the fridge and pre-warm to room temperature. If bubbles are present in the PeptiGel®, centrifuge the vial containing PeptiGel® at 1,600 x *g* for 1 minute, repeat if required.

3. Using a positive displacement pipette, transfer 1 mL of PeptiGel® into a 15 mL Falcon tube.

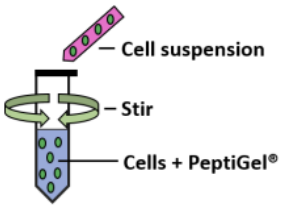


4. Resuspend your cells in up to 200 µL of cell culture medium.

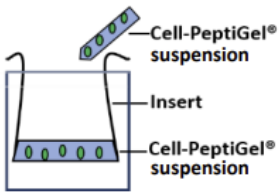
   Note: A concentrated cell suspension is required to achieve the target cell densities as your cell suspension will subsequently be diluted in 1 mL of PeptiGel®.



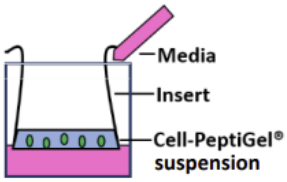
5. Transfer the cell suspension to the bottom of the tube containing 1 mL of PeptiGel® and carefully mix. To ensure a homogenous mixture, insert the pipette to the bottom of the gel and release the cells slowly whilst gradually bringing the pipette upwards, in a stirring motion. To avoid bubbles, make sure the pipette tip never leaves the hydrogel while mixing.



6. Pipette 50 – 100 µL of the hydrogel-cell mixture into the inserts. The volume will depend on the desired thickness.



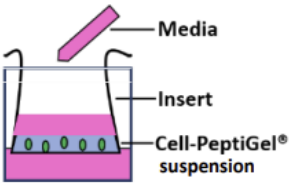
7. Add 1000 µL of cell culture medium to each well around the insert.



8. Incubate at 37°C for 5 minutes.

9. Add 250 µL of cell culture medium carefully to the top of the inserts and return the plate to incubate at 37°C.

   Note: To avoid any evaporation boundary effect, add PBS or water to any empty wells.



10. Change the cell culture medium on the top an bottom of the insert 2-3 times within 1 hour. Incubate at 37°C between the medium changes, then incubate at 37°C overnight.

    Note: When changing the cell culture medium, leave some medium on the surface of the PeptiGel® each time to prevent the pipette tip disrupting the PeptiGel®.

11. After 24 hours, replace the cell culture medium. Repeat the cell culture medium replacement depending on the requirements of your cell type.

For further support, please contact our technical support team at tech@cellgs.com.

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